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(54) Title: QUALITATIVE ULTRAVIOLET TEST FOR DETECTION OF ETHOXYQUIN

(57) Abstract: A method of quickly and qualitatively measuring levels of ethoxyquin within samples of vegetable or animal products or vitamin products in the field by extracting ethoxyquin into a solution and observing under an ultraviolet light. The solution contains a water-immiscible organic solvent, a salt, water, and optionally a polar solvent. A positive indication of the presence of ethoxyquin is a visually observed color when the solution is observed under an ultraviolet light.

# QUALITATIVE ULTRAVIOLET TEST FOR DETECTION OF ETHOXYQUIN

## BACKGROUND OF INVENTION

This application claims priority to U.S. provisional patent application Ser. No. 60/141,153 filed June 25, 1999.

### Field of the Invention

The present invention generally relates to a field test for ethoxyquin with minimal sample preparation and quick, on-site results that can be readily performed by shipping, quality control, and/or receiving personnel to qualitatively verify the presence of ethoxyquin in vegetable or animal products.

### Description of Related Art

Animal Feed ingredients such as fats and vitamins bring a value to animal diets. Fats in animal feed supply energy for both maintenance and growth of the animals. They provide essential fatty acids for the synthesis of prostaglandins. They also facilitate the adsorption of fat soluble vitamins and enhance flavor. In addition, fats reduce dust in feed mills during the manufacture of animal feed and at grow-out houses.

Oxidation can significantly reduce many of the benefits provided by the addition of fats and vitamins and can also introduce damaging by-products. For example, oxidation consumes energy in the fat and that energy is then unavailable to an animal. Oxidation begins when a carbon-hydrogen bond in a fat or vitamin breaks to produce a chemical species called a free radical. This free radical reacts with the oxygen in air to produce compounds called peroxides. Peroxides are unstable products and continue to react further to give a

wide variety of by-products including aldehydes, ketones, alcohols, esters, acids and polymers.

Some of the by-products from oxidation, especially the aldehydes, can be toxic to the animal. Fat soluble vitamins can be destroyed leading to deficiency syndromes such as steatitis and encephalomalacia. Damage can be seen even at the cellular level due to the reaction with peroxides or the toxicity of the aldehydes produced as by-products of the oxidation process. As a consequence of that oxidation, an animal will not reach its full potential and the net result to the producer is poorer financial return due to lower body weight and poorer feed conversion. These problems can be minimized with good quality control which begins with monitoring the quality of the ingredients received at rendering plants and feed mills.

In 1988, Cabel and Waldroup reported on a study with male broilers fed four diets containing specified levels of oxidized fat. The control diet was prepared with unoxidized fats. Cabel, M.C., et al., 1988, Effects of Ethoxyquin Feed Preservative and Peroxide Level on Broiler Performance, Poultry Science, 67:1725-1730. The other three diets were prepared from oxidized fats and formulated to contain 2, 4 and 7 meq. of peroxides per kg of feed, respectively. A trend to poorer body weight was observed as the level of peroxides increased. The birds fed the diets containing 7 meq peroxides/kg of feed had significantly lower body weight than the birds fed the control diet. There was also a trend to poorer feed conversion as the peroxide content of the diets increased. The negative effects of oxidation were mitigated by the inclusion of ethoxyquin in the diets prepared with oxidized fat.

A similar study was conducted by Dr. Schang and coworkers using rancid meat meal with identical results.

These studies confirm that feeding oxidized fats can cost the grower money. Schang, M.J., et al., 1987, Quality of Ingredients and Their Effect on Growth: Rancidity, 10th LATAM Poultry Congress, Buenos Aires, Argentina, 29 September - 2 October, 221.

A three-week feeding study was conducted by Dibner et al. using oxidized fat to try to understand what were the underlying reasons that produced the poorer body weight and feed conversions reported by Cabel et al. (1988) and by Schang et al. (1987). Dibner, J.J., et al., 1996, Feeding of Oxidized Fats to Broilers and Swine: Effects on Enterocyte Turnover, Hepatocyte Proliferation and the Gut Associated Lymphoid Tissue, Animal Feed Science and Technology, 62:1-13. Four diets were used in the study. Two of these included fresh fat with and without ethoxyquin. The other two diets were prepared with or without ethoxyquin using 4% fat that had been oxidized to 100 meq of peroxides. This produced diets that had 4 meq peroxides per kg of feed. The same effects of reduced body weight and reduced feed conversion were seen in this study as seen by Cabel and by Schang. The birds fed the diets containing oxidized fat plus 125 ppm of ethoxyquin had significantly better feed to gain than those birds consuming the diets prepared with the oxidized fat without ethoxyquin. The birds on the control diets performed better than those birds consuming oxidized fat. But even here, there was a trend to better performance in the diet with ethoxyquin even when those diets were prepared with fresh fat. This was evident as early as 14 days with a significantly better feed to gain for those birds consuming the fresh fat with ethoxyquin compared with those birds consuming either of the two diets prepared with the oxidized fat. An intermediate level of performance was observed for those birds consuming the control diet, but without the

added ethoxyquin. Of particular interest was the observation that the trends could be seen already within seven days, although the differences were not yet significant at that time.

The poorer body weight and feed conversion are the external effects that can be seen by the grower. Several factors were observed in the study at the cellular level that could contribute to those measurable results. First, the pattern of concentrations of red blood cells was exactly the same as the body weight. The highest concentration was in the birds fed the fresh fat plus ethoxyquin and the lowest concentration was in the birds fed the diets prepared from oxidized fat without added ethoxyquin. Second, consuming the diets prepared with oxidized fat and no ethoxyquin caused a transient reduction of the Lactobacilli in the microflora of the gut. With the reduced Lactobacilli, the E. coli grew heavily. Third, the birds fed the diets with the oxidized fat had measurably increased liver cell and intestinal epithelial cell turnover showing the impact of the peroxides and the toxic by-products from oxidation in these tissues. Finally, there was a reduced level of IgA in the gut of the birds fed the oxidized fat suggestive of reduced immune response.

Oxidation can be controlled through the use of an antioxidant such as ethoxyquin. Ethoxyquin, or 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, is a chemical that is used by rendering plants, animal processing plants, and feed mills as a preservative or stabilizer in a variety of animal and vegetable products. Ethoxyquin is a free radical trap. It can intercept the free radicals to stop the formation of peroxides and prevent the formation of the other by-products. This enables the ethoxyquin to control the oxidation process, limit the

damage from the free radicals and limit the development of the toxic by-products.

Ethoxyquin is used in a variety of animal feed ingredients and products such as animal and vegetable products, vitamins and vitamin premixes to preserve the materials and prevent oxidative degeneration. The animal and vegetable products produced by rendering plants include such materials as poultry meal, meat and bone meal, fish meal, poultry fat, animal fat, tallow, lard, yellow grease made from animal or vegetable oils and fats, and other by-product meals (hereinafter referred to as Arendered products@). The rendering plants may add ethoxyquin to their products at any period during the processing of the animal or vegetable materials. The rendered products are then shipped to feed mills for incorporation into animal feeds.

Animal processing plants also add ethoxyquin to waste animal oils and fats that are removed from machines, floors, and waste water in order to comply with environmental regulations. These waste animal oils and fats are termed diffused air floatation (DAF) sludge. The DAF sludge is then shipped to rendering plants as a raw material to also be incorporated with other raw materials for processing into rendered products.

Vitamins and vitamin premixes (hereinafter referred to as Avitamin products@) that are added to animal feed also contain ethoxyquin as an ingredient to stabilize the vitamin products.

The problems associated with oxidation of fats can therefore be minimized by ensuring the presence of ethoxyquin in the DAF sludge, rendered products, or vitamin products and by monitoring the quality of the ingredients received at rendering plants and feed mills. Stabilization of the high fat ingredients is a key step in maintaining quality.

As shipments of DAF sludge are received by rendering plants or rendered products are received by feed mills, the only way to currently determine whether the products contain ethoxyquin is to conduct a chemical analysis on a sample. This requires that a sample be analyzed at either the receiving plant=s in-house laboratory or to ship a sample to an off-site analytical laboratory to determine the levels of ethoxyquin using known analytical methods. This process is time consuming, requiring overnight analysis for in-house laboratories or several days or weeks for shipping and analysis at off-site laboratories.

Rendering plants, feed mills, and the shipping trucks delivering the materials to these plants do not have time to wait overnight or a week or two to receive analytical results before accepting the shipped products or having the products mixed in feed. A need has therefore been identified to develop a screening tool that can be used to quickly determine whether rendered products, DAF sludge or vitamin products are stabilized with ethoxyquin. Such a method is needed as only a screening tool to determine the presence of ethoxyquin and not to quantify the ethoxyquin levels in the materials.

While the benefits of ethoxyquin have been long known, no method has been available that would allow rendering plant or feed mill personnel to test for an antioxidant in the DAF sludge, rendered products, vitamins or vitamin premixes before they could be unloaded from the truck or railroad car into the storage bin.

Three qualities increase the value of an ethoxyquin detection method to a rendering plant, processing plant, or feed mill. The first is ease of use; ideally the method can be conducted by an employee without technical

training. The second is the time required to obtain the results of the detection method; ideally the time is sufficiently short that a truck driver can conveniently wait for the analysis to be completed before unloading the truck and putting the ingredient into the storage tanks. The third is expense; ideally, the method is inexpensive. If it is too expensive, the rendering plants and feed mills may not spend the money to do it.

## SUMMARY OF INVENTION

Accordingly, it is an object of the present invention to provide a quick, qualitative method in which the presence of ethoxyquin is able to be screened in a material of animal or vegetable origin, or vitamin product processed by rendering plants and animal processing plants, and manufacturers of feed vitamins.

Briefly, therefore, the present invention is directed to a method in which a sample of material of animal or vegetable origin, or vitamin product is combined with a solvent to form an extract. A mixture is formed from a salt, water, a water-immiscible organic solvent, and the extract. The water-immiscible organic solvent is then exposed to an ultraviolet light and visually inspected for the presence of a purple/blue color. The presence of a purple/blue color qualitatively indicates the presence of ethoxyquin in the sample.

In another aspect, the invention is directed to a method in which a sample of material of animal or vegetable origin, or vitamin product is mixed with a polar solvent thereby forming a first extraction mixture. A second extraction mixture is formed from a salt, water, a water-immiscible organic solvent, and the polar solvent from the first extraction mixture. The second extraction mixture is then exposed to a ultraviolet light and visually inspected to determine whether it emits a color

in the water-immiscible organic layer. The presence of a purple/blue color qualitatively indicates the presence of ethoxyquin in the sample without further quantifying the concentration of ethoxyquin.

In a further aspect, the invention is directed to a method in which a sample of material of animal or vegetable origin, or vitamin product is combined with a water-immiscible organic solvent thereby forming a first extraction mixture. A second mixture is formed from a salt, water, and the water-immiscible organic solvent from the first extraction mixture. The second extraction mixture is then exposed to a ultraviolet light and visually inspected for the presence of a purple/blue color in the water-immiscible organic layer. The presence of a purple/blue color qualitatively indicates the presence of ethoxyquin in the sample.

In still a further aspect, the invention is directed to a method in which a sample of material of animal or vegetable origin, or vitamin product is combined with a water-immiscible organic solvent, a salt, and water to form an extraction mixture. The extraction mixture is then exposed to a ultraviolet light and visually inspected for the presence of a purple/blue color in the water-immiscible organic layer. The presence of a purple/blue color qualitatively indicates the presence of ethoxyquin in the sample.

Other features of the present invention will be in part apparent to those skilled in the art and in part pointed out in the detailed description provided below.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Rendering plant and feed mill personnel would benefit from having an analytical method for ethoxyquin that can quickly determine if ethoxyquin was added to the incoming ingredients before a truck or railroad car

containing rendered products, DAF sludge, or vitamin products is unloaded. This represents AReal Time@ analysis.

The present invention (Athe qualitative method@) has been developed to qualitatively detect the presence of ethoxyquin in rendered products, DAF sludge, and vitamin products. This test can be run frequently because it is easy, inexpensive and rapid enough to be used by personnel such as rendering plant and feed mill employees. More frequent analyses will provide more control over the suppliers and better quality control of feed ingredients and the feed produced from them. This should ultimately lead to better animal performance (e.g. less feed is required to increase animal body weight) and better profits.

Ethoxyquin fluoresces a purple/blue color when placed under a ultraviolet light. Previous analytical methods have used this property to quantitatively determine ethoxyquin concentrations in materials through the use of a fluorometer. These prior methods required substantial effort by experienced laboratory personnel. They could not be carried out by employees without technical training at the shipping and receiving docks. In contrast, using the qualitative method, one can qualitatively determine if a solution contains ethoxyquin by placing the solution under a ultraviolet light and visually observing, without additional instrumentation or sample processing, the presence or absence of a fluorescing purple/blue color.

The qualitative method is designed to extract ethoxyquin from rendered products, DAF sludge or vitamin products by using a simple extraction procedure. The presence of ethoxyquin is detected by observing the solution under an ultraviolet light. If the sample fluoresces, it contains ethoxyquin. If it does not, the

sample contains no more than about 50 ppm ethoxyquin. The general detection limit is normally 50 ppm, although lower levels may be detectable in some ingredient matrices.

The method involves four steps: (1) extract a sample with a solvent, (2) add salt, water in a mixture that also contains a water-immiscible organic solvent, and shake. The top layer is (3) observed under an ultraviolet light, and (4) if it has a purple/blue glow, ethoxyquin is present in the material.

Rendering plant products, DAF sludge, and vitamin products can therefore be tested for the presence of ethoxyquin by extracting any ethoxyquin present in the product into solution. This can be achieved by mixing rendered products, DAF sludge, or vitamin products with a single portion of a solvent for a period of time and then allowing the mixture to settle. The volume or weight of the sample does not have to be precisely measured because the qualitative method is not designed to provide a quantitative number. A large spoon such as a tablespoon or soup spoon can be used to measure the sample (e.g. about 5 g or 15 ml).

About 1.5 parts of sample by volume (e.g. about 5 g or 15 ml) is placed in a beaker or a similar container with about 5 parts solvent (e.g. about 15 ml sample is mixed with about 50 ml solvent). The solvent is preferably a polar solvent, although a water-immiscible organic solvent may also be used. Examples of polar solvents that may be used include, but are not limited to, methanol, ethanol, isopropyl alcohol, acetonitrile, and acetic acid. Examples of water-immiscible organic solvents include, but are not limited to, petroleum ether, toluene, chloroform, and alkanes (such as pentanes, hexanes, octanes, isooctane, cyclohexane). The sample is preferably shaken or stirred for about 2

minutes. Mechanical stirring with a magnetic stirrer is convenient to use, or the container can be sealed and shaken by hand.

A fraction of the solvent portion of the mixture is then removed (e.g. approximately 1 part or about 10 ml). The sample residue need not be extracted further with fresh portions of the solvent. The removed fraction of the solvent is then poured into a disposable plastic tube or similar container and thoroughly mixed together with water (e.g. approximately 1 part solvent by volume per 3 parts water or about 10 ml solvent per 30 mL water), a sufficient quantity of salt to give a 1.5% to 10% salt concentration when mixed with the water, and a water-immiscible organic solvent (e.g. approximately 1 part or about 10 mL of water-immiscible organic solvent). Salts which may be used are inorganic salts, examples of which include, but are not limited to, salts of alkali metal and alkali earth metal (such as sodium chloride, potassium chloride, magnesium sulfate, and any monovalent or divalent inorganic salt). Examples of water-immiscible organic solvents include, be are not limited to, petroleum ether, toluene, chloroform, and alkanes (such as pentanes, hexanes, octanes, isooctane, cyclohexane).

The tube is preferably sealed and shaken for about 15 seconds. The salt and water act to break up any emulsion between the water-miscible layer and water-immiscible organic solvent layer by providing an ionic source to separate the layers. If ethoxyquin is present in the sample, it will be pulled into the water-immiscible organic solvent.

The water-immiscible organic solvent layer is then allowed to separate from the mixture into its own layer after the mixture has been shaken. The water-immiscible organic solvent layer is viewed under an ultraviolet

light, preferably a long-wavelength ultraviolet light emitting a wavelength between 355 nm to 375 nm, most preferably around 366 nm. If ethoxyquin was present in the original sample in concentrations of approximately 50 ppm or more, one can visually observe the water-immiscible organic solvent fluorescing a purple/blue color. Otherwise, the sample will simply be an unfluorescing liquid.

Positive results for the qualitative test for ethoxyquin can be detected in concentration levels as low as 25 ppm to 50 ppm, depending on variations in sources of tested materials. The color may be visually observed under normal indoor lighting, or more preferably, a more pronounced color may be visually observed if the non-ultraviolet lights are dimmed.

The preferred embodiment is to mix about 1.5 parts of the sample by volume with about 5 parts methanol (e.g. approximately 15 mL or 1 tablespoon sample is mixed with about 50 mL methanol) to form a first extraction mixture. The sample is shaken or stirred for about 2 minutes. A fraction of the methanol portion of the mixture is then removed (e.g. approximately 1 part or about 10 mL). The removed methanol fraction from the first extraction mixture is combined with 3 parts water (e.g. approximately 1 part solvent by volume per 3 parts water or about 10 mL solvent per 30 mL water), a quantity of sodium chloride to give a 1.5% to 10% sodium chloride concentration when mixed with the water, and approximately 1 part petroleum ether (e.g. about 10 mL of petroleum ether) to form a second extraction mixture. The tube is sealed and shaken for about 15 seconds. The petroleum ether layer is then allowed to separate from the second extraction mixture into its own layer after the mixture has been shaken. A sample of the petroleum ether is removed and viewed under a long-wavelength

ultraviolet light emitting a wavelength between 355 nm to 375 nm, most preferably around 366 nm. If ethoxyquin was present in the original sample in concentrations of approximately 50 ppm or more, one can visually observe the water-immiscible organic solvent fluorescing a purple/blue color.

The test may be simplified without using a polar solvent. Similar to the steps above, a large spoon such as a tablespoon or soup spoon can be used to measure the sample. About 1.5 parts of sample by volume (e.g. about 5 g or 15 mL) is placed in a beaker or a similar container with about 5 parts of a water-immiscible organic solvent (e.g. about 15 mL sample is mixed with about 50 mL water-immiscible organic solvent) to form a first extraction mixture. The first extraction mixture is shaken for about 2 minutes in a beaker or similar container. A fraction of the solvent portion of the mixture is then removed (e.g. approximately 1 part or about 10 mL). A second extraction mixture is formed by combining the removed fraction of the water-immiscible organic solvent, water (e.g. approximately 1 part water-immiscible organic solvent by volume per 3 parts water or about 10 mL water-immiscible organic solvent per 30 mL water), and a sufficient quantity of salt to give a 1.5% to 10% salt concentration when mixed with the water. The second extraction mixture is preferably shaken or stirred for about 15 seconds. The liquid layers are allowed to separate. The water-immiscible organic solvent layer is then visually observed under an ultraviolet light. If ethoxyquin is present, the water-immiscible organic solvent layer will visually appear purple/blue in color.

The test may be simplified even further by preparing an extraction mixture consisting of about 3 parts water (e.g. about 30 mL), a sufficient quantity of salt to give a 1.5% to 10% salt concentration when mixed

with the water, about 1.5 parts of the sample (approximately 5 g or 15 mL), and about 5 parts water-immiscible organic solvent (e.g. approximately 50 mL). The extraction mixture is preferably shaken or stirred for about 2 minutes. The liquid layers are allowed to separate and the extraction mixture is exposed to an ultraviolet light. If ethoxyquin is present, the water-immiscible organic layer will visually appear purple or purple/blue in color.

The qualitative method can utilize a variety of solvents and salts to achieve the desired qualitative test results. Reagent grade solvents and salts provide the best test results as they lack impurities that can cause interferences in the test.

The hardware necessary to fluoresce the sample solution is an ultraviolet light, preferably a long-wavelength ultraviolet light emitting wavelengths in the range of 355 nm to 375 nm, most preferably about 366 nm.

In using the qualitative method for ethoxyquin, rendering plants, processing plants, and feed mills will be able to quickly detect the presence of ethoxyquin in rendered products, DAF sludge, and vitamin products prior to being shipped or received by the plants or mills while they have the products sent out for quantitative analysis. In this way, the method promotes improved quality of rendered products, DAF sludge, vitamin products and finished feed by confirming in Areal time@ that rendered products, DAF sludge, and vitamin products contain ethoxyquin when specified. Finally, this method brings added value to ethoxyquin products, permitting the preservative to be tested for continued effectiveness in the materials it is intended to preserve.

The invention method is not designed to provide the same degree of precision as the official method for the measurement of ethoxyquin. The invention method is

qualitative. The standard analytical method published by the AOAC should be used if exact levels of ethoxyquin are required. Tables I & II compare the qualitative method and AOAC method for detecting ethoxyquin. The qualitative method has a minimum detection level of 50 ppm, and sometimes as low as 25 ppm, depending on the sample source materials; whereas the official method can be used down to levels of 20 ppm.

The real advantage of the qualitative method regards the time and cost savings of running the tests. The qualitative method takes only 3 to 5 minutes per analysis whereas the AOAC method takes a minimum of 2 hours to complete. Non-technical personnel can easily be trained to run the invention method at rendering plants or feed mills. Typically, the official procedure cannot be run at rendering plants or feed mills because it requires a trained technician, who is located either in the company=s central lab or in a commercial lab. This means that the time required for the AOAC method of analysis may be extended to days while the samples are transported to the lab site.

The cost savings are primarily a combination of a reduction in time for an employee to run the test plus a lower cost for items consumed during the analysis. The cost to have a employee at a rendering plant or feed mill run a test in 5 minutes is much less than the cost of a two hour analysis run by a technician in a laboratory. The on-site analysis also eliminates any shipping charges.

TABLE I

Method Comparisons in Commercial Fats

| Sample | AOAC Official Method for Ethoxyquin (ppm) 1 | Qualitative Test for Ethoxyquin2 |
|---|---|---|
| Poultry Fat | 231 | + |
| Lard | 342 | + |
| Yellow Grease | 162 | + |

1 AOAC Official Method 963.07, Official Methods of Analysis of AOAC International, 16th Ed. 1998:4.10.02, Chapter 4, p. 43.

2 A positive test result (i.e. a visual blue/purple color) is indicated by a "+".

TABLE II

Method Comparisons in Commercial By-Product Meals

| Sample | AOAC Official Method for Ethoxyquin (ppm)[1] | Qualitative Test for Ethoxyquin[2] |
|---|---|---|
| Poultry Meal | 107 | + |
| Poultry Meal | 230 | + |
| Meat Meal | 143 | + |
| Meat Meal | 122 | + |

1 AOAC Official Method 963.07, Official Methods of Analysis of AOAC International, 16th Ed. 1998:4.10.02, Chapter 4, p. 43.

2 A positive test result (i.e. a visual blue/purple color) is indicated by a "+".

EXAMPLE 1 - Qualitative Test for Ethoxyquin Using a Polar Solvent

Materials

Methanol, petroleum ether and sodium chloride, all commercially available reagent grade chemicals. Magnetic stirrer and stir bars, glass beakers, glass test tubes, and long wavelength (366 nm) ultraviolet light, all commercially available. Deionized or tap water.

Method

Add approximately 5 grams of sample (fat or meal) and 50 mL of methanol to a glass beaker. Stir manually or mechanically (e.g. with a magnetic stirrer) for 2 minutes. Allow solid material to settle.

Decant 10 mL of supernatant to another beaker, add 30 mL of a 1.5 to 10% salt water solution and 10 mL petroleum ether and shake for 15 seconds. Allow mixture to settle undisturbed and separate into polar and water-immiscible organic solvent layers.

Visually examine the top organic layer color (petroleum ether) under a long-wavelength ultraviolet light. Alternatively, decant top organic layer (petroleum) to a test tube and visually examine the water-immiscible organic solvent's color under a long-wavelength ultraviolet light. The presence of a fluorescing purple/blue color is a positive response for the presence of ethoxyquin.

The responses will vary between sources of the sampled products. The qualitative detection level of ethoxyquin can be detected in some samples down to 25 ppm.

EXAMPLE 2 - Qualitative Test for Ethoxyquin Without a Polar Solvent in Two Extraction Mixtures

Materials

Petroleum ether and sodium chloride, all commercially available reagent grade chemicals. Magnetic stirrer and stir bars, glass beakers, glass test tubes, and long-wavelength (366 nm) ultraviolet light, all commercially available. Deionized or tap water.

Method

Add 5 grams of sample (fat or meal) and 50 mL of petroleum ether to a glass beaker. Stir manually or mechanically (e.g. with a magnetic stirrer) for 2 minutes. Allow solid material to settle.

Decant 10 mL of supernatant to another beaker, add 30 mL of a 1.5 to 10% salt water solution and shake for 15 seconds. Allow mixture to settle undisturbed and separate into salt water and water-immiscible organic solvent layers.

Visually examine the top organic layer color (petroleum ether) under a long-wavelength ultraviolet light. Alternatively, decant the top organic layer (petroleum ether) to a test tube and visually examine the color under a long-wavelength ultraviolet light. The presence of a fluorescing purple/blue color is a positive response for the presence of ethoxyquin.

The responses will vary between sources of the sampled products. The qualitative detection level of ethoxyquin can be detected in some samples down to 25 ppm.

EXAMPLE 3 - Qualitative Test for Ethoxyquin Without a Polar Solvent in One Extraction Mixture

Materials

Petroleum ether and sodium chloride, all commercially available reagent grade chemicals. Magnetic stirrer and stir bars, glass beakers, glass test tubes, and long-wavelength (366 nm) ultraviolet light, all commercially available. Deionized or tap water.

Method

Add approximately 5 grams of sample (fat or meal), 50 mL of petroleum ether, 30 mL of a 1.5 to 10% salt water solution to a glass beaker. Stir manually or mechanically (e.g. with a magnetic stirrer) for 2 minutes.

Allow mixture to settle undisturbed and separate into salt water and water-immiscible organic solvent layers.

Visually examine the top organic layer color (petroleum ether) under a long-wavelength ultraviolet light. Alternatively, decant the top organic layer (petroleum ether) to a test tube and visually examine the color under a long-wavelength ultraviolet light. The presence of a fluorescing purple/blue color is a positive response for the presence of ethoxyquin.

The responses will vary between sources of the sampled products. The qualitative detection level of ethoxyquin can be detected in some samples down to 25 ppm.

As various changes could be made in the above method without departing from the scope of the invention, it is intended that all matter contained in the above description be interpreted as illustrative and not in a limiting sense.

We Claim:

1. A method for detecting the presence of ethoxyquin in a material of animal or vegetable origin, or vitamin product comprising:

combining the material with a solvent to form an extract;

forming a mixture comprising the extract, a salt, water, and a water-immiscible organic solvent;

exposing water-immiscible organic solvent from the mixture to an ultraviolet light; and

visually inspecting the water-immiscible organic solvent as it is being exposed to the ultraviolet light for the presence of a color which qualitatively indicates the presence of ethoxyquin in the sample.

2. The method of claim 1 wherein the material of animal or vegetable origin, or vitamin product is selected from the group consisting of poultry meal, meat and bone meal, fish meal, poultry fat, animal fat, tallow, lard, yellow grease made from animal or vegetable oils and fats, by-product meals, vitamins and vitamin premixes, and DAF sludge.

3. The method of claim 1 wherein the water-immiscible organic solvent is selected from the group consisting of petroleum ether, toluene, chloroform, and alkanes.

4. The method of claim 3 wherein the alkanes are selected from the group consisting of pentane, hexane, octane, isooctane, and cyclohexane.

5. The method of claim 1 wherein the solvent is selected from the group consisting of methanol, ethanol, isopropyl alcohol, acetonitrile, and acetic acid.

6. The method of claim 1 wherein the salt is an inorganic salt selected from the group consisting of alkali metals and alkali earth metals.

7. The method of claim 6 wherein the inorganic salt is selected from the group consisting of sodium chloride, potassium chloride, and magnesium sulfate.

8. The method of claim 1 wherein the ultraviolet light is a long wavelength light emitting a wavelength in the range of 355 nm to 375 nm.

9. The method of claim 1 wherein the solvent used to form the extract is methanol, the water-immiscible organic solvent is petroleum ether, and the salt is sodium chloride.

10. The method of claim 1 wherein the solvent used to form the extract is a water-immiscible organic solvent.

11. A method for detecting the presence of ethoxyquin in a material of animal or vegetable origin, or vitamin product comprising:

forming a first extraction mixture which comprises a sample of the material and a polar solvent;

forming a second extraction mixture which comprises a salt, water, a water immiscible organic solvent, and polar solvent from the first extraction mixture;

exposing the water immiscible organic solvent from the second extraction mixture to an ultraviolet light;

and

visually inspecting the water immiscible organic solvent as it is being exposed to the ultraviolet light for the presence of a purple/blue color which qualitatively indicates the presence of ethoxyquin in the sample.

12. The method of claim 11 wherein the material of animal or vegetable origin, or vitamin product is selected from the group consisting of poultry meal, meat and bone meal, fish meal, poultry fat, animal fat, tallow, lard, yellow grease made from animal or vegetable oils and fats, by-product meals, vitamins and vitamin premixes, and DAF sludge.

13. The method of claim 11 wherein the water-immiscible organic solvent is selected from the group consisting of petroleum ether, toluene, chloroform, and alkanes.

14. The method of claim 13 wherein the alkanes are selected from the group consisting of pentane, hexane, octane, isooctane, and cyclohexane.

15. The method of claim 14 wherein the polar solvent is selected from the group consisting of methanol, ethanol, isopropyl alcohol, acetonitrile, and acetic acid.

16. The method of claim 15 wherein the salt is selected from the group consisting of salts of alkali metal and alkali earth metal.

17. The method of claim 16 wherein the salt is selected from the group consisting of sodium chloride,

potassium chloride, and magnesium sulfate.

18. The method of claim 17 wherein the ultraviolet light is a long wavelength light emitting a wavelength in the range of 355 nm to 375 nm.

19. The method of claim 18 wherein the polar solvent is methanol, the water-immiscible organic solvent is petroleum ether, and the salt is sodium chloride.

20. A method for detecting the presence of ethoxyquin in a material of animal or vegetable origin, or vitamin product comprising:

forming a first extraction mixture which comprises a sample of the material and a water-immiscible organic solvent;

forming a second extraction mixture which comprises a salt, water, and water-immiscible organic solvent from the first extraction mixture;

exposing the second extraction mixture to a ultraviolet light; and

visually inspecting the second extraction mixture as it is being exposed to the ultraviolet light for the presence of a color which qualitatively indicates the presence of ethoxyquin in the sample.

21. The method of claim 20 wherein the material of animal or vegetable origin, or vitamin product is selected from the group consisting of poultry meal, meat and bone meal, fish meal, poultry fat, animal fat, tallow, lard, yellow grease made from animal or vegetable oils and fats, by-product meals, vitamins and vitamin premixes, and DAF sludge.

22. The method of claim 21 wherein the water-immiscible organic solvent is selected from the group consisting of petroleum ether, toluene, chloroform, and alkanes.

23. The method of claim 22 wherein the alkanes are selected from the group consisting of pentane, hexane, octane, isooctane, and cyclohexane.

24. The method of claim 23 wherein the salt is selected from the group consisting of alkali metals and alkali earth metals.

25. The method of claim 24 wherein the salt is selected from the group consisting of sodium chloride, potassium chloride, and magnesium sulfate.

26. The method of claim 25 wherein the ultraviolet light is a long wavelength light emitting a wavelength in the range of 355 nm to 375 nm.

27. The method of claim 21 wherein the water-immiscible organic solvent is petroleum ether and the salt is sodium chloride.

28. A method for detecting the presence of ethoxyquin in a material of animal or vegetable origin, or vitamin product comprising:

forming an extraction mixture which comprises a sample of the material, a salt, water, and water-immiscible organic solvent;

exposing the extraction mixture to an ultraviolet light; and

visually inspecting the extraction mixture as it is being exposed to the ultraviolet light for the presence

of a color which qualitatively indicates the presence of ethoxyquin in the sample.

29. The method of claim 28 wherein the material of animal or vegetable origin, or vitamin product is selected from the group consisting of poultry meal, meat and bone meal, fish meal, poultry fat, animal fat, tallow, lard, yellow grease made from animal or vegetable oils and fats, by-product meals, vitamins and vitamin premixes, and DAF sludge.

30. The method of claim 29 wherein the water-immiscible organic solvent is selected from the group consisting of petroleum ether, toluene, chloroform, and alkanes.

31. The method of claim 30 wherein the alkanes are selected from the group consisting of pentane, hexane, octane, isooctane, and cyclohexane.

32. The method of claim 31 wherein the salt is selected from the group consisting of alkali metals and alkali earth metals.

33. The method of claim 32 wherein the salt is selected from the group consisting of sodium chloride, potassium chloride, and magnesium sulfate.

34. The method of claim 33 wherein the ultraviolet light is a long wavelength light emitting a wavelength in the range of 355 nm to 375 nm.

35. The method of claim 29 wherein the water-immiscible organic solvent is petroleum ether, and the salt is sodium chloride.

# INTERNATIONAL SEARCH REPORT

Intern[illegible]plication No
PCT/US 00/16354

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, INSPEC, COMPENDEX, IBM-TDB, BIOSIS, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | S.C. WITT ET AL.: "Simplified method for the determination of ethoxyquin in alfalfa products and mixed feeds" JOURNAL OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, vol. 56, no. 1, 1973, pages 167-170, XP000961315 abstract page 167, right-hand column, line 1 - line 5 | 1-5,8, 10-15, 18, 20-23, 26, 28-31,34 |
| A | page 167, right-hand column, last paragraph -page 168, left-hand column, line 31 | 9,19,27, 35 |

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 November 2000 | 21/11/2000 |

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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Thomas, R.M. |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| Y | A. GUYOT: "Étude des traceurs de la matière grasse butyrique. 5. L'éthoxyquine." BULLETIN DES RECHERCHES AGRONOMIQUES DE GEMBLOUX, vol. 8, no. 1, 1973, pages 3-18, XP000961409 page 6, line 1 - line 3 page 9, last paragraph -page 10, line 2 page 12, last paragraph -page 13, line 2 | 1-5,8, 10-15, 18, 20-23, 26, 28-31,34 |
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